Application Note 02110

Direct Aqueous Injection and Automated On-Line Solid Phase Extraction (SPE) of Phenoxy Herbicides in Drinking Water Using the Varian 320-MS Triple Quadrupole LC/MS/MS

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Introduction

Phenoxy herbicides are members of a class of chemicals related to the growth hormone indoleacetic acid. Contact with broad-leaf plants induces rapid, uncontrolled growth, killing the affected plants. Phenoxy herbicides are widely used to selectively destroy broad-leaved weeds amongst wheat or corn crops.

The best-known phenoxy herbicides are 2,4-dichlorophenoxy-acetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T, shown in Figure 1). 2,4,5-T itself is only moderately toxic, with oral LD_{50} of 389 mg/kg. However, the manufacturing process for 2,4,5-T contaminates this chemical with trace amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is reported to be extremely toxic to humans. 2,4-D has not been reported as being contaminated with TCCD, however it is one of the most widely used herbicides in the world and is a regulated drinking water contaminant in the United States and other countries.

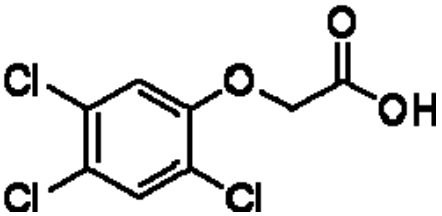


Figure 1. Molecular structure of the phenoxy herbicide 2,4,5-trichloropenoxyacetic acid (2,4,5-T).

The trace-level quantitation of herbicides in drinking water requires analytical speed, selectivity, and sensitivity. Fully on-line automated SPE analysis provides sample preparation and detection in a single step, saving time and labor for the analyst. In addition, the "closed" nature of an on-line system prevents contamination of the sample and/or losses due to evaporation. Further, in on-line SPE analysis, the complete sample — rather than an aliquot of the sample — is analyzed, which improves the ability for detection of the analyte. As a result, water sample sizes are reduced, saving sample shipping costs and storage space.

A method is detailed below using the Varian 320-MS LC/MS/MS to provide automated direct aqueous injection and on-line SPE for routine surveillance of 17 acid herbicides in drinking water. A limit of detection (LOD) of less than 20 ng/L for all compounds studied is reported with a five point linear calibration from 20 to 500 ng/L.

Reproducibility over 10 injections at less than 11% RSD is obtained at the routine surveillance level of 100 ng/L. These typical results demonstrate the performance of 320-MS LC/MS/MS method for monitoring drinking water.

Instrumentation

- Varian 320-MS LC/MS/MS with ESI Source (Varian Part Number 0293795212)
- Varian 212-LC Binary Solvent Delivery Modules (Varian Part Number LCMSHPLC02)
- Varian ProStar™ 210 Loading Pump (Varian Part Number 0393550001)
- 5 mL Ti Pump Head for 210 Pump (Varian Part Number 0393594091)
- 8700 PSI Ti Pressure Module (Varian Part Number 0393552501)
- Varian ProStar 410 AutoSampler (Varian Part Number 0393529001)
- Varian ProStar 510 Column Oven (Varian Part Number 0393572102)

Sample Preparation

Samples were prepared in a North Oxfordshire drinking water matrix. Sodium thiosulfate (18 mg) was added to 1 L of tap water. Samples were made up in this water and the water acidified with 0.1% with formic acid.

HPLC Conditions

Trapping Column: Pursuit™ XRs C18, 5 µm, 50 x 2.0 mm (Varian Part Number A6000050X020)

Analytical Column: Polaris™ C18, 3 µm, 100 x 2.0 mm (Varian Part Number A2001100X020)

Varian 212-LC Pumps:

Solvent A: 0.02% formic acid in water

Solvent B: 0.02% formic acid in acetonitrile

Varian ProStar 210 Pump:

Solvent C: 0.1% formic acid in water

Programs:

Analytical LC:

Time (min:sec)	%A	%B	Flow (mL/min)
00:00	100	0	0.250
07:00	100	0	0.250
17:00	0	100	0.250
21:00	0	100	0.250
21:06	100	0	0.250
26:00	100	0	0.250

Loading Pump LC:

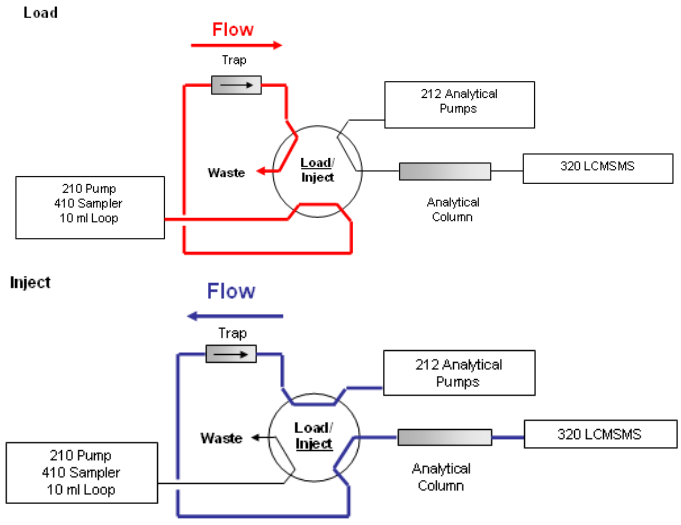
Time (min:sec)	%C	Flow (mL/min)
00:00	100	1.0
10:00	100	1.0
10:10	100	0.0
25:00	100	1.0
26:00	100	1.0

Divert Valve Program:

Time (min:sec)	Load/Inject
00:00	Load
06:00	Inject
21:00	Load

Injection Volume: 10 mL

Flow Diagram



MS Parameters

Ionization Mode: ESI Negative
API Drying Gas: 18 psi at 200 °C
API Nebulizing Gas: 65 psi
Detector: 1550 V
Needle: -4500 V
Shield: -600 V
CID Gas: 1.5 mTorr

Results and Discussion

This LC/MS/MS method separates and quantifies 17 phenoxy herbicides at trace levels using a 26-minute run time.

Figures 2 and 3 show overlaid chromatograms of 2,4-D and bentazone at concentrations ranging from 20 to 500 ng/L.

Table 2. 320-MS segment parameters.

Compound	MS/MS Transitions	Capillary Voltage (V)	Collision Energy (V)
2,3,6-TBA	223 → 179 225 → 181	-30 -30	7 7
Dicamba-d_3	224 → 180	-30	6.5
Dicamba	219 → 175	-30	6.5
Benazolin	242 → 170 242 → 198	-30 -30	12.5 5.5
Fluroxypyr	253 → 196 253 → 233	-30 -30	12 6
Bentazone-d_6	245 → 132	-30	25
Bentazone	239 → 132 239 → 197	-30 -30	25 19
$^{13}C_6$ 2,4-D	225 → 167	-30	12.5
2,4-D	219 → 161 219 → 125	-30 -30	12.5 25
MCPA-d_6	205 → 147	-30	15
Bromoxynil	276 → 276	-30	3
MCPA	199 → 141 199 → 155	-30 -30	15 8.5
2,4-DP-d_6	239 → 164	-30	15
2,4-DP	233 → 161	-30	15
Ioxynil	370 → 126 370 → 370	-30 -30	22 4.5
MCPP	213 → 141 215 → 143	-30 -30	15 15
2,4,5-T	253 → 195	-30	10
2,4-DB	247 → 161	-20	7.5
MCPB	227 → 141	-20	10
2,4,5-TP	267 → 195	-20	10
Dichlorophen	267 → 127 267 → 267 269 → 127	-30 -30 -30	19.5 7 19.5
$^{13}C_6$ PCP	271 → 271 273 → 273	-30	7 7
PCP	263 → 263 265 → 265 267 → 267	-30	7 7 7

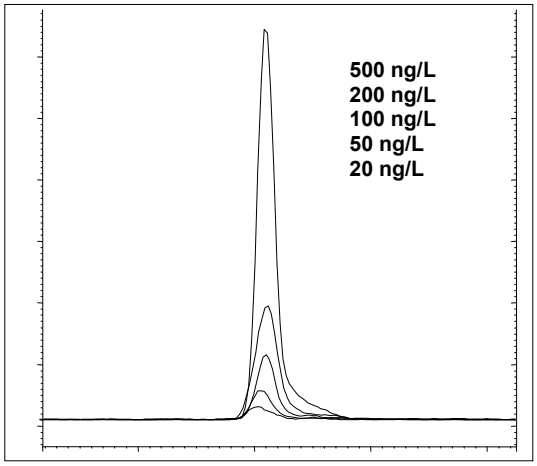


Figure 2. Overlaid chromatogram showing injections of 2,4-D from 20 to 500 ng/L with the automated on-line SPE technique.

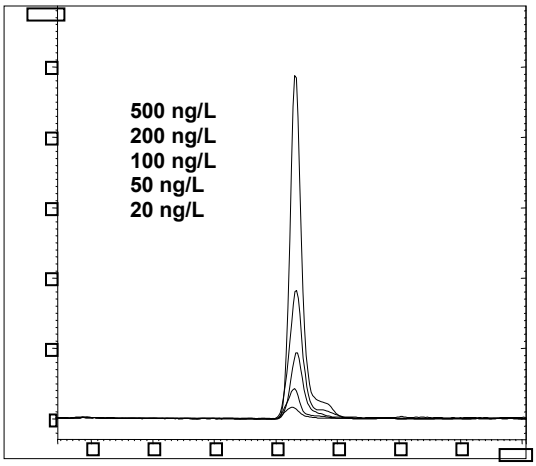


Figure 3. Overlaid chromatogram showing injections of bentazone from 20 to 500 ng/L.

Calibration curves for all analytes were found to be linear from 20 to 500 ng/L. Figure 4 shows the calibration curve for bentazone, with an r^2 value equal to 0.9997 from 20 to 500 ng/L. Table 3 shows the r^2 values for all calibration curves as well as the RSD values for 10 replicate injections at 100 ng/L.

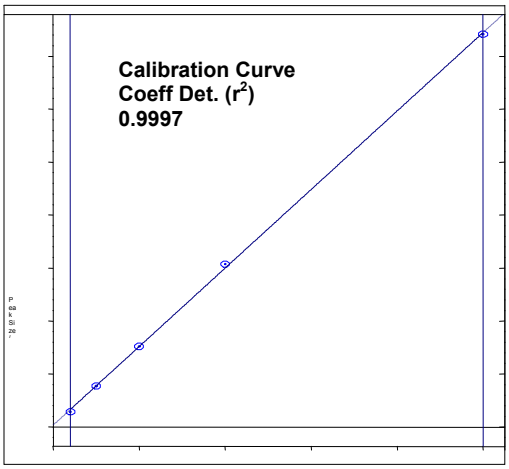


Figure 4. Standard calibration curve for bentazone on the 320-MS, r^2 = 0.9997.

Table 3. Calibration curve r^2 values and %RSD values for 10 replicate injections.

Compound	10 Replicate %RSD	Calibration Curve (r^2)
2,3,6-TBA	7.54	0.9978
Dicamba	10.42	0.9993
Benazolin	6.72	0.9992
Fluroxypyr	10.59	0.9989
Bentazone	5.44	0.9997
2,4-D	6.15	0.9994
Bromoxynil	8.91	0.9993
MCPA	6.17	0.9993
2,4-DP	5.15	0.9999
Ioxynil	7.99	0.9977
MCPP	5.83	0.9995
2,4,5-T	7.97	0.9992
2,4-DB	5.44	0.9998
MCPB	10.49	0.9984
2,4,5-TP	6.31	0.9989
Dichlorophen	5.62	0.9991
PCP	4.51	0.9987

Conclusion

This method, including on-line SPE and MS/MS provides an accurate and sensitive analysis for phenoxy herbicides. The calibration curves were found to be linear from 20 to 500 ng/L with r^2 values ranging from 0.9977 to 0.9999, and 10 replicate injections at 100 ng/L produced %RSD values ranging from 4.51% to 10.59%. The on-line technique reduces labor, sample handling, and provides very precise and accurate results.

These data represent typical results.
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